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XANTHINE OXIDASE AS A MARKER OF MYOCARDIAL INFARCTION

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ABSTRACT

In the present communication, we report remarkably elevated levels of xanthine oxidase activity in the blood of the patients with myocardial infarction when compared to age and sex matched healthy persons. Highly significant increase of malondialdehyde, serving as an index of lipid peroxidation and thus free radical mediated damage, has also been found in the patients. We propose the measurement of the blood levels of xanthine oxidase, a very simple, reliable and less time consuming method as an indicator of myocardial infarction.

KEY WORDS

Xanthine oxidase, Myocardial infarction, Biochemical marker, Malondialdehyde.

INTRODUCTION

For the assessment of the myocardial damage various biochemical markers, namely aspartate amino transferase (AST), lactate dehydogenase isoenzymes (LDH) and creatine kinase isoenzymes (CK-MB) are used. These markers are of not much value in the assessment of myocardial injury, because of certain limitations (1). There are reports to show that abnormal levels of troponin are found in various conditions which are not related to acute coronary diseases (2). Search for new biochemical markers for myocardial damage is, therefore, desirable.

Oxygen free radical generation has been shown to be an important mechanism of cellular injury in ischemic myocardium (3). Several mechanisms have been proposed to be involved in the generation of oxygen free radicals but xanthine oxidase has been shown to be a major source of free radical generation under ischemic conditions (4). Xanthine oxidase is widely distributed, occurring in milk, small intestine, kidney and liver. The enzyme is involved in the metabolism of purines, catalyzing the conversion of both hypoxanthine and xanthine to uric acid. Probable role of the enzyme in milk is it's antibacterial activity by virtue of it's ability to produce oxygen derived free radicals. Oxy free radicals produced by the action of xanthine oxidase, in turn, oxidize cellular proteins and membranes resulting in myocardial cellular injury (5).

Xanthine oxidoreductase, under normal conditions, exists in dehyrogenase form and uses NAD^+ and there is no or very little production of superoxide anion (3). Under ischemic conditions, there is depletion of ATP and subsequent loss of membrane Ca^{2+} gradient. Increased Ca^{2+} levels activates Ca^{2+} dependent proteases which cause selective proteolysis of the dehydrogenase to convert it into xanthine oxidase which acts both on hypoxanthine and xanthine at the expense of molecular oxygen to produce superoxide ion (6,7). Thus xanthine oxidase in ischemic conditions of the heart, as in myocardial infarction, may play an important role in contributing free radical mediated damage.

In the present study we have measured the levels of xanthine oxidase and malondialdehyde in patients with myocardial infarction and compared with age and sex matched healthy persons serving as control.

MATERIALS AND METHODS

All the biochemicals were procured from M/s Sigma Chemical Company USA. Other chemicals were of analytical grade of Qualigens or equivalent. The patients group comprised of 115 patients with myocardial infarction. Clinical examination of all patients was done to detect any co-existing disorder. Cases with known organic disorders were excluded from the study.

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All the cases were subjected to routine investigation e.g., hematocrit, urine analysis and blood chemistry (urea, creatinine, lipids, sugar, Na^+ and K^+) and electrocardiogram. Hundred age and sex matched group of healthy persons served as control. Both healthy and patient groups comprised of non-smokers and within the age group of 40-65 years. Informed consent was taken from each patient and healthy person employed in the study. The study was cleared by departmental ethical committee.

Collection of Blood Sample : Venous blood (1 ml.) was withdrawn and transferred into citrated vials. The blood was suitably diluted for analysis wherever required.

Assay of Xanthine Oxidase Activity : Assay of xanthine oxidase was carried out essentially according to the method described by Roussos (8). The assay mixture, in final volume of 3.0 ml, consisted of 0.30 ml Tris-HCl buffer, 50 mM pH 7.4; 0.30 ml $CuSO_4$, 10 mM ; 0.05 ml. Xanthine , 2.58 mM per ml. in 0.05 M glycine buffer, pH 7.4; 0.1 ml. of diluted blood and water to make up the volume . Change in absorbance was recorded at 290 nm at 15 seconds interval for one minute. Suitable control was run simultaneously. One unit of activity has been defined as change in absorbance at 290 nm in 1 minute by 1 ml. enzyme preparation.

Protein estimation : Protein was estimated by the method of Lowry et al. (9) using Folin phenol reagent. Bovine serum albumin was used as the standard.

Measurement of Lipid per oxidation : Lipid peroxidation was estimated by the method of Ohkawa et al. (10) by measuring the levels of MDA. To 0.20 ml. of blood was added 0.8 ml. sodium dodecyl sulphate, 8.1%; 0.50 ml glacial acetic acid and thiobarbituric acid, 0.8% to make up the volume to 3.0 ml. Contents of the tubes were mixed and heated over water bath maintained at 90°C for one hour and immediately cooled thereafter under running water. To each tube, 1.0 ml. water and 5.0 ml solution of n-butanol and pyridine (15:1 v/v) was added and vortexed thoroughly and centrifuged at 800×g for ten minutes. The upper layer was aspirated out and color intensity measured at 532 nm. The reference used was 1, 1, 3, 3 tetra ethoxy propane.
Statistical analysis were carried out using Student's 't' test.

RESULTS

As shown in the table, specific activity of xanthine oxidase increases by 400% in the blood of patients with myocardial infarction when compared to healthy persons ($p<0.0005$).

Xanthine oxidase and MDA levels in the blood of healthy persons and patients with myocardial infarction

Cases	Specific activity of Xanthine oxidase (units/mg protein)	MDA (nmole×10^6/ ml blood)
Healthy persons (n=100)	0.0096±0.008	29.79±1.43
Patients with myocardial Infarction (n=115)	0.0479±0.0024	55.87±1.52
p value	P<0.0005	P<0.0005

Values reported as mean±SD; n = no. of cases.
P<0.0005 highly significant.

Malondialdehyde (MDA) levels are also statistically very significantly increased in the blood of patients with myocardial infarction. There is about 88% increase in MDA levels in the blood of patients when compared to healthy controls ($p<0.0005$).

DISCUSSION

Xanthine oxidase is an important source of free radical generation (11,12). During ischemic conditions, the adenosine nucleotide pool is degraded to hypoxanthine and xanthine, along with conversion of xanthine dehydrogenase to xanthine oxidase (6,7). Xanthine oxidase acts on xanthine and hypoxanthine with the resultant production of oxygen free radicals.

Highly significant increase ($p<0.0005$) in the activity of xanthine oxidase in the blood of patients with myocardial infarction indicates that myocardial ischemia has a definite correlation with xanthine oxidase activity and thus the measurement of xanthine oxidase activity may be used as a biochemical marker of myocardial infarction along with electrocardiographic observations.

Free radicals are extremely reactive and consequently short lived, therefore, their activity is usually assessed by indirect methods such as measurement of various end products resulting from interaction of free radicals with cellular components such as lipids, proteins and DNA. Study of interaction of free radicals with lipids can be readily carried out to assess free radical mediated damage. Lipids when react with free radicals, they undergo per-oxidation to form lipid per-oxides. Lipid per-oxides decompose to form numerous products including malondialdehyde. Measurement of malondialdehyde levels is the most popular and easiest used indicator of lipid per-oxidation and subsequently free radical

reactivity in biological samples.

Highly significant levels of MDA ($p<0.0005$) in the blood of patients with myocardial infarction has been found and it may also be used as the marker of the ischemic myocardial syndrome, but increase in MDA levels is much less than that of xanthine oxidase. Moreover, the measurement of MDA levels is complicated and time consuming as compared to the assay of xanthine oxidase activity.

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